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TLR3: A Receptor that Recognizes Cell Injury Is Essential for Permeability Barrier Homeostasis Following UV Irradiation

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The cutaneous permeability barrier is essential for life and perturbations in this barrier are repaired rapidly. After minimal injury to the stratum corneum alterations in the calcium concentration in the outer epidermis are the primary signal inducing this repair response. In this issue, studies demonstrate that Toll-like receptor 3 has an important role in signaling permeability barrier repair following injury induced by UVB irradiation.

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The most important function of the skin is to form a barrier between the hostile outside world and the internal milieu. The skin must protect the body from outside hazards such as pathogenic microorganisms, mechanical insults, toxic chemicals, UVR, etc. In addition, the skin must prevent the loss of water and electrolytes from the body in order to maintain homeostasis. The permeability barrier, which inhibits water and electrolyte movement, is localized to the stratum corneum and is mediated by unique extracellular lipid membranes containing cholesterol, free fatty acids, and ceramides (Feingold, 2007; Feingold and Elias, 2014). Marked derangements in this permeability barrier, which can occur following severe burns or in premature infants, are not compatible with life outside of intensive care units. Milder derangements in the permeability barrier are seen in neonates, the elderly, and in association with many common skin disorders including atopic dermatitis and psoriasis (Feingold, 2007; Feingold and Elias, 2014). In fact, recent studies have linked perturbations in the permeability barrier with the development and progression of atopic dermatitis and psoriasis (Wolf and Wolf, 2012; Wolf *et al.*, 2012).

Given the importance of the permeability barrier for life, a large number of studies have been carried out to determine how this barrier is maintained. One can perturb the barrier by mechanical disruption (tape stripping), treating the surface of the skin with solvents (for example acetone), or treating the surface of the skin with detergents (for example SDS) all of which remove the extracellular lipid membranes that mediate permeability barrier function (Feingold, 2007; Feingold and Elias, 2014). These manipulations result in a marked increase in transepidermal water loss, which is rapidly restored toward normal such that within 24–48 hours the rate of transepidermal water loss returns to baseline levels, indicating normal barrier function (Feingold, 2007; Feingold and Elias, 2014). In order to repair the barrier there is a coordinated response in the underlying epidermis, one that restores the extracellular lipid membranes in the stratum corneum (Feingold, 2007; Feingold and Elias, 2014). Specifically, a preformed pool of lamellar bodies is secreted rapidly by stratum granulosum cells into the extracellular spaces of the stratum corneum. This is followed by an increase in *de novo* synthesis of cholesterol, fatty acids, and ceramides by keratinocytes. In addition, fatty acid transport proteins and lipoprotein receptors increase on the cell surfaces of keratinocytes, facilitating the uptake of extracutaneous lipids. The increase in keratinocyte lipids allows for the rapid assembly and secretion of new lamellar bodies that can restore the extracellular lipid membranes in the stratum corneum. There is also an increase in the synthesis of the proteins that are carried in lamellar bodies, most notably the enzymes that are required for the extracellular processing of lipids to form mature lamellar membranes. Thus, the epidermis has a complex coordinated response that reestablishes the integrity of the cutaneous permeability barrier (Feingold, 2007; Feingold and Elias, 2014).

An important question concerns the identity of the signals that initiate this rapid repair response following acute injury. To begin, the outer epidermis contains a high concentration of calcium. Immediately following barrier disruption, there is an increased movement of water through the stratum corneum that transports calcium and other ions, leading to a marked reduction in the calcium concentration surrounding the keratinocytes in the outer epidermis. This triggers the barrier repair response (Feingold and Denda, 2012). If the calcium concentration in the outer epidermis is maintained following permeability barrier disruption, rapid lamellar body secretion does not occur and the abnormally high transepidermal water loss is not repaired (Feingold and Denda, 2012). Conversely, if the calcium content of the outer epidermis is lowered by iontophoresis or sonophoresis, without disrupting the permeability barrier, lamellar body secretion is still initiated (Feingold and Denda, 2012). It is likely that parallel changes in potassium and perhaps other ions are also important in signaling permeability barrier repair. In addition, permeability barrier disruption results in the increased expression of several cytokines (tumor necrosis factor, IL-1, IL-6, and others) and growth factors (vascular endothelial

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Clinical Implications

- The cutaneous permeability barrier is essential for life, and therefore injury induces a rapid repair response that quickly restores it.
- Minimal injuries to the stratum corneum that perturb the permeability barrier result in alterations in the calcium concentration in the outer epidermis, which is the primary signal for barrier repair.
- Following more severe injury that is induced by UVB irradiation, Toll-like receptor 3 has an important role in mediating permeability barrier repair.

growth factor, nerve growth factor, amphiregulin), and studies in mice deficient in either these cytokines/growth factors or their receptors have demonstrated a delay in permeability barrier repair, indicating a role for these signaling molecules in facilitating the repair process, as well (Feingold and Denda, 2012).

It is essential to recognize that the above described studies have focused exclusively on injuries that were localized predominantly to the stratum corneum (i.e., minimal injury). It is well recognized that more severe injuries to the skin will also disrupt the permeability barrier. For example, exposure to high doses of UVR has been shown to lead to a marked increase in transepidermal water loss, which returns toward normal over several days (Haratake *et al.*, 1997; Holleran *et al.*, 1997). The signaling pathways that restore the permeability barrier following more severe injuries have been unknown, but in this issue of the *Journal of Investigative Dermatology*, Richard Gallo's laboratory now addresses this important question.

Earlier studies by the Gallo laboratory have demonstrated that epidermal injury leads to an increase in double stranded RNA that can bind to Toll-like receptor 3 (TLR3), a receptor that is part of the innate immune system (Bernard *et al.*, 2012). Although activation of TLR 3 is well recognized to have a key role in immunity against viral infections, more recent studies have demonstrated that this receptor is also activated after cell injury. Furthermore, activation of TLR3 has been shown to stimulate the expression of several key genes that have a role in permeability repair, including ABCA12, glucocerebrosidase, acid sphingomyelinase, serine palmotyltransferase, glucosylceramide synthase, and transglutaminase 1 (Borkowski *et al.*, 2013). Most importantly, activation of TLR3 increases the number of lamellar bodies in keratinocytes, suggesting that activation of the innate immune system stimulates permeability barrier repair (Borkowski *et al.*, 2013). In this issue, Gallo and colleagues first demonstrate that the products of UVR B-damaged keratinocytes increase the expression of ABCA12, glucocerebrosidase, acid sphingomyelinase, and transglutaminase 1 (Borkowski *et al.*, 2014). They next demonstrate that in keratinocytes multiple non coding small nuclear RNAs that increase in number following UVB exposure also increase the expression of key permeability barrier repair proteins. Most importantly, they show that the ability of mice to repair their permeability barrier after UV irradiation is delayed in TLR3-deficient mice. Of particular note is that permeability barrier repair following barrier disruption by tape stripping or chemical depilation (minimal injuries) was not altered in TLR 3–deficient mice, demonstrating that the role of TLR3 appears to require significant tissue injury. Finally, transplanting normal bone marrow cells into TLR3-deficient mice did not restore permeability barrier homeostasis to normal in response to UVR treatment, demonstrating that TLR3 on keratinocytes is likely required for normal permeability barrier repair. However, TLR −/− bone marrow transplanted into normal animals worsened permeability barrier homeostasis, indicating that bone marrow–derived cells have at least some role in permeability barrier repair following UVR treatment.

As with all studies, many questions remain. What are the specific pathways by which activation of TLR3 accelerates permeability barrier repair? What is the role of TLR3 on keratinocytes and on bone marrow–derived cells in regulating permeability barrier homeostasis? Are there other injuries to the permeability barrier that also require TLR3 for its repair? What are the specific activators of TLR3 following UVR-induced permeability barrier injury? Are these activators increased following other cutaneous injuries? Does TLR3 have a role in the defective permeability function seen in cutaneous disorders such as atopic dermatitis or psoriasis? One can expect that over the next several years these and other questions will be answered and that our understanding of permeability barrier homeostasis will be increased even further.

CONFLICT OF INTEREST

The author states no conflict of interest.

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